WHAT IS CLAIMED:

1. A nucleic acid molecule comprising a low homology packaging signal cassette flanked by a recombinase recognition sequence, wherein said packaging signal cassette comprises a modified adenovirus packaging signal, provided that said modified packaging signal has low homology to a wild-type adenovirus packaging signal.

2. The nucleic acid of claim 1, wherein said recombinase recognition sequence is *loxP*.

3. The nucleic acid of claim 1, wherein said recombinase recognition sequence is *frt*.

4. The nucleic acid of any one of claims 1-3, wherein said modified packaging signal is less efficient than said wild-type packaging signal.

5. The nucleic acid of claim 4, wherein said wild-type packaging signal is human adenovirus serotype 5 packaging signal.

6. The nucleic acid of claims 5, wherein the modified packaging signal comprises at a maximum, 23 bp of contiguous sequence homology with said wild-type packaging signal.

7. The nucleic acid of claim 5, wherein said modified packaging signal is about 2-3 times less efficient than said wild-type signal.

8. The nucleic acid of claim 6, wherein said modified packaging signal comprises two to six A elements, each A element having a consensus sequence of ATTTGN8GC.

9. The nucleic acid of claim 6, wherein said nucleic acid is a plasmid.

10. The nucleic acid of claim 6, wherein said nucleic acid is a helper virus.

11. The nucleic acid of claim 10, wherein said helper virus does not contain an E1 gene.

12. The nucleic acid of claim 11, wherein said helper virus comprises an E3 region with an insert of about 2.9 kb.

13. The nucleic acid of claim 12, wherein said insert does not contain a promoter sequence.

14. A nucleic acid comprising an adenovirus E3 gene having an insertion of at least about 2.7 kb, provided that said insertion does not contain a promoter sequence.

15. The nucleic acid of claim 14, wherein said insertion is a human intron sequence.

16. An adenoviral helper virus for production of helper dependent vectors comprising:

(a) an adenovirus genome having an E1 region deletion;

(b) an excisable packaging signal cassette replacing a wild-type packaging signal, the excisable packaging signal cassette comprising a 5' *loxP* site, a modified packaging signal and a 3' *loxP* site, wherein said modified packaging signal has low homology to and is less efficient than the wild-type packaging signal; and

(c) an optional insertion element comprising at least about 2900 base pairs of non-adenoviral DNA inserted in the E3 region without deleting any part of the E3 region.

17 . The virus of claim 16 wherein said adenovirus genome is human Adenovirus type 5.

18. The virus of claim 17 wherein said modified packaging signal comprises at a maximum, 23 bp of contiguous sequence homology with said wild-type packaging signal.

19. The virus of claim 18, wherein said modified packaging signal is about 2-3 times less efficient than said wild-type signal.

20. The virus of claim 19, wherein said modified packaging signal comprises two to six A elements, each A element having a consensus sequence of ATTTGN8GC.

21. A vector comprising the adenovirus of claim 16.

22. A cell line expressing E1 and infected with the helper virus of claim 16.

23. The cell line of claim 22, wherein said cell line further expresses *cre* recombinase.

24. The cell line of claim 23, wherein said cell line is 293 *cre* cells.

25. A helper-dependent adenovirus vector comprising:

a) a 5' ITR;

b) a packaging signal;

c) at least one heterologous expression cassette;

d) a human genomic stuffer DNA;

e) an optional E4 non-coding segment conferring a selective advantage, wherein the E4 element is located between nucleotide -400 from the right end; and

f) a 3' ITR;

wherein the overall size of the vector is between about 28 kb and 36 kb, and wherein the only adenoviral sequences present are the ITRs, optional E4 non-coding segment and the packaging signal, and wherein no bacterial origin of replication or bacterial marker genes are present.

26. The helper dependent adenovirus vector of claim 25, wherein said optional E4 element is present.

27. A plasmid vector comprising

a) a 5' ITR;

b) a packaging signal;

c) at least one heterologous expression cassette;

d) a human genomic stuffer DNA;

e) an optional E4 non-coding segment conferring a selective advantage, wherein the E4 element is located between nucleotide -400 from the right end; and

f) a 3' ITR.

28. A helper-dependent adenovirus comprising in a 5' to 3' direction:

a) a 5' ITR,

b) a packaging signal cassette directly joined to the 3' of said 5' ITR,

c) a first stuffer DNA at least about 1 kb,

d) at least one heterologous expression cassett,

e) a second stuffer DNA at least about 1 kb,

f) an optionally present non-coding E4 segment at least 400 bp in length; and

g) a 3' ITR, wherein said 3' ITR is directly joined to the 5' end of said non-coding E4 segment if present;

provided that said helper-dependent adenoviral vector does not encode one or more proteins needed for viral generation, is about 28 kb to about 36 kb, and has a GC content between about 50% and about 60%.

29. The helper-dependent adenoviral vector of claim 28, wherein said virus is between 30 and 36 kb in length.

30. The helper-dependent adenoviral vector of claim 29, wherein said first stuffer and said second stuffer are derived from inverted mammalian non-gene or intron sequences.

31. The helper-dependent adenoviral vector of claim 30, wherein said virus does not encode for any adenovirus proteins.

32. The helper-dependent adenoviral vector of any one of claims 28-31, wherein said optionally present non-coding E4 region is present.

33. The helper dependent virus of claim 32, wherein said GC content is between 52% to 57%.

34. A method of generating helper-dependent adenoviral gene vectors in a cell line expressing E1 and *cre* recombinase comprising:

a) infecting said cell line with a helper-dependent vector comprising: a 5' ITR, a packaging signal, at least one heterologous expression cassette, human genomic stuffer DNA and a 3' ITR, wherein the overall size of the helper-dependent vector is between about 28 kb and 36 kb, and wherein no functional adenoviral coding sequences and no bacterial origin of replication or bacterial marker genes are present;

b) infecting the cell line with a helper virus comprising: an adenovirus genome having an E1 region deletion; an excisable packaging signal cassette replacing a wild-type packaging signal, the excisable packaging signal cassette comprising a 5' *loxP* site, a modified packaging signal and a 3' *loxP* site, wherein the modified packaging signal has low homology to and is less efficient than the wild-type packaging signal; and an optional insertion element comprising at least about 2900 base pairs of non-adenoviral DNA inserted in the E3 region without deleting any part of the E3 region; and

c) obtaining the generated helper-dependent viral vectors.

35. A method of generating a helper-dependent adenoviral vector comprising:

a) producing a cell comprising

(i) *trans* functions needed for adenovirus generation; and

(ii) said helper-dependent adenoviral vector, wherein said helper-dependent adenoviral vector comprises the necessary *cis* functions needed for

adenovirus generation and at least one heterologous expression cassette, and said helper-dependent adenoviral vector does not encode for any adenovirus proteins, is about 28 kb to about 36 kb, and has a GC content between about 50% and about 60%, and

b) generating said helper-dependent adenoviral vector.

36. The method of claim 35, wherein late proteins and either E2 proteins or E4 proteins, or both E2 proteins and E4 proteins are supplied by a helper virus present in said cell.

37. The method of claim 36, wherein said helper virus comprises a low homology packaging signal cassette flanked by a recombinase recognition sequence, wherein said packaging cassette signal comprises a modified adenovirus packaging signal having low homology to a wild-type adenovirus packaging signal.

38. The method of claim 37, wherein said recombinase recognition sequence is *loxP* and said cell expresses Cre recombinase.

39. The nucleic acid of claim 37, wherein said recombinase recognition sequence is *frt* and said cell expresses Flp recombinase.

40. The method of claim 37, wherein said modified packaging signal is less efficient than said wild-type signal.

41. The method of any one of claims 36-40, wherein said wild-type packaging signal is from human adenovirus serotype 5.